Electronic Supplementary Material (ESI) for Food & Function.
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Supplementary materials

The supplementary file containing Supplementary eTables 1-2, Supplementary eMethods 1-2, Supplementary eFigure 1, and Reference are provided.

Supplementary eTables:

eTable 1. List of the *C.elegans* strains used in this study.

eTable 2. List of the primers used in this study for qPCR analysis.

Supplementary eMethods:

eMethod 1. The specific process of synchronization and administration in *C.elegans*.

eMethod 2. The detailed protocol of widely targeted metabolome method.

eTable 1. List of the *C.elegans* strains used in this study.

Strain name	Genotype
N2	Wild-type Bristol
IR1511	N2;Ex001[p_{myo-3} DsRed::LGG-1;p_{dct-1} DCT-1::GFP]
DA2123	adIs2122 [*lgg-1p*::GFP::*lgg-1* + *rol-6*(su1006)]
TJ356	zIs356 [*daf-16p*::*daf-16a/b*::GFP + *rol-6*(su1006)]
RW1596	stEx30 [*myo-3p*::GFP::*myo-3* + *rol-6*(su1006)]
SJ4103	zcIs14 [*myo-3*::GFP(mit)]
CF1038	*daf-16*(*mu86*) I.
TK22	*mev-1*(*kn1*) III.

The information presented in this table is provided from Caenorhabditis Genetics Center and Prof. Tavernarakis at the University of Crete.

eTable 2. List of the primers used in this study for qPCR analysis.

Gene name	Primer sequences	
	Forward	**Reverse**
Reference gene		
pmp-3	GTTCCCGTGTTCATCACTCA	ACACCGTCGAGAAGCTGTAGA
Mitochondrial proteostasis		
ubl-5	ATGATTGAAATCACAGTAAA	TCATTGGTAGTAGAGCTCGA
clpp-1	GGCAGTGTAACAGCAGGACT	GGCGCTCATGAAACGATCAC
haf-1	TTGGAACCGTTGGTCAGGAG	CCACTTGGTCAGCTTGTTGC
hsp-16.2	GGAACGCCAATTTGCTCCAG	AGATTCGAAGCAACTGCACC
hsp-6	ACAGGCCGTTACCAACTCTG	TGTTGACGGTGGTTCCCAAA
hsp-60	AGGGATTCGAGAGCATTCGTCAAG	TGTGGCGACTTGAGCGATCTCTTC
atfs-1	CAATCACCATCAAAATCGGCG	CTTGCTCAATGTCCATTTCGAAC
lonp-1	CATGTACCCGAAGGAGCAA	GCGATCACTTTTTCCCGAAT
Mitochondrial biogenesis		
aak-2	TCTTCCGCCATCCGCATATC	CCTCTTCATCGGGTCTACGC
sir-2.1	CGATGCACCCGAAACAAACA	TTCTGCCTTACAGGAGCACG
skn-1	TCAACCGTCCAATGGGTCTC	GTGCCCTTCTCTCCAGCAAT
hmg-5	CGTCCAAGTGTTCCTCCAAGTG	CTTCGCTTCGTCTGTGTACTTCTTT
nhr-65	TGGACGAAATGCTTGGCTTG	ACGTTGAAAAGCTCCGCGAT
Mitochondrial dynamics		
phb-2	GGAGGATTGTCGACGGATTT	GTTTGGTCTGGCACGGATA
drp-1	AGCCCACCAATGAGCTTGTC	GAGCACTGACCGCTCTTTCT
fis-1	CTCGACTTTCATACCGAACAGG	CTCCGATCCTCTGATCCAACTA
fzo-1	GACAGGTGCCGACCTTATG	CTCTGGAACGCTCATTCTTTCT
eat-3	TGATGCGTTTAGAGCAGCCA	TGAAGAAGCATACGCAGGCA
anc-1	CGGTTCACAAAGGACGAGTAT	CCGAGTACGATACGTGGATTTC
Mitophagy		
dct-1	TGGTATGTCAGAATCGTGGGTG	ACGGACAGTCTTTGGAGGTG
pdr-1	AGCCACCGAGCGATTGATTGC	GTGGCATTTTGGGCATCTTCTTG
pink-1	AGCATATCGAATCGCAAATGAGTTAG	TCGACCGTGGCGAGTTACAAG
lgg-1	CGTGCCGAAGGAGACAAGAT	CTTCCTCGTGATGGTCCTGG
rheh-1	GGCTCCAACCTTACCACTCC	GCAAATCCTACT GCTGCTCC
ced-9	AAAGGCACAGAGCCCACC	CGTTCCCATAACTCGCATC
bec-1	ACGAGCTTCATTCGCTGGAA	TTCGTGATGTTGTACGCCGA
unc-51	CTACACGTGGTGACTCTCCG	ATGCAATACGACGCGAAAGC
daf-16	GAGGAGCACAGCTTCCAGAAT	ATTGAGCTCCGCCTCCAATG

eMethod 1. The specific process of synchronization and administration in *C.elegans*.

Synchronization: the young adult worms with intensive eggs were cleaned and collected by M9 buffer, then lysed with alkaline lysate for 5 min. The alkaline lysate consists of 50% 10-fold dilutions of sodium hypochlorite solution (NaOCl, 6~14% active chlorine basis, Macklin) and 50% 1 M sodium hydroxide solution. After centrifugation and washing 2~3 times with M9 to remove the lysis solution, their eggs were transferred into blank NGM plates and developed into L4 larvae at 20°C.

Administration: after 20 min under UV exposure, 90 mm NGM-plates with sufficient food (*E. coli* OP50) were followed by the addition of the corresponding concentration of M9 buffer or drug solution (400 μL) on the surface and placed at 4°C for storage after air-drying on super-clean table. The three iridoids or extract are water soluble to use directly dissolved in M9 buffer, while UA was dissolved in a DMSO stock solution. The corresponding concentration of DMSO at 1% final that not affect nematode physiological functions.

eMethod 2. The detailed protocol of widely targeted metabolome method.

The experiment set a total of three groups: normal aging, high-fat exacerbated aging, and 100 μM Asp administration under high-fat exacerbated aging. Each group of about 2500 nematodes was continuously cultured until 9 days and then collected, washed completely, and extracted the mitochondrial fraction. 200 μM FUDR in the NGM medium was used to inhibit progeny production. In combination with the BCA kit (Kang century Biotechnology Co., China), a final sample of 50 μL 0.5 μg protein equivalents/μL per group was obtained for subsequent metabolomics.

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD, https:// sciex.com.cn/; MS, QTRAP® System, https://sciex.com/). The analytical conditions were as follows, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 × 100 mm); column temperature, 40°C; flow rate, 0.4 mL/min; injection volume, 2μL; solvent system, water (0.1% formic acid): acetonitrile (0.1% formic acid); gradient program, 95:5 V/V at 0 min, 10:90 V/V at 11.0 min, 10:90 V/V at 12.0 min, 95:5 V/V at 12.1 min, 95:5 V/V at 14.0 min.

LIT and triple quadrupole (QQQ) scans were acquired on a triple quadrupole-linear ion trap mass spectrometer (QTRAP), QTRAP® LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in positive and negative ion mode and controlled by Analyst 1.6.3 software (Sciex). The ESI source operation parameters were as follows: source temperature 500°C; ion spray voltage (IS) 5500 V (positive), -4500 V (negative); ion source gas I (GSI), gas II (GSII), curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively; the collision gas (CAD) was high. Instrument tuning and mass calibration were performed with 10 and 100 μM polypropylene glycol solutions in QQQ and LIT modes, respectively. A specific set of MRM transitions were monitored for each period according to the metabolites eluted within this period.

Unsupervised principal component analysis (PCA) was performed by statistics function prcomp within R (www.r-project.org). The data was unit variance scaled before unsupervised PCA.

The hierarchical cluster analysis (HCA) results of metabolites were presented as heatmaps with dendrogram. And HCA was carried out by R package Complex-Heatmap. For HCA, normalized signal intensities of metabolites (unit variance scaling) are visualized as a color spectrum.

Differential metabolites selected: For two-group analysis, differential metabolites were determined by VIP (VIP > 1) and *P*-value (*P*-value < 0.05, Student's t test). VIP values were extracted from OPLS-DA result, which also contain score plots and permutation plots, was generated using R package MetaboAnalystR. The data was log transform (log_2) and mean centering before OPLS-DA. In order to avoid overfitting, a permutation test (200 permutations) was performed.

KEGG annotation and enrichment analysis: Identified metabolites were annotated using KEGG Compound database (http://www.kegg.jp/kegg/ compound/), annotated metabolites were then mapped to KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html). Pathways with significantly regulated metabolites mapped to were then fed into metabolite sets enrichment analysis (MSEA), their significance was determined by hypergeometric test's *p*-values. The Rich Factor is the ratio of the number of differential metabolites in the corresponding pathway to the total number of metabolites annotated to that pathway, which a higher value indicating greater enrichment.